

## Molecular phylogenetics of six marine microalgae strains from Greek transient waters

Koletti<sup>1</sup> A., Efroze<sup>1,2</sup> R. C., Chantzistrountsiou<sup>3</sup> X., Papadaki<sup>3</sup> S., Tzovenis<sup>3</sup> I., Economou-Amilli<sup>3</sup> A., Flemetakis<sup>1</sup> E.

<sup>1</sup>Agricultural University of Athens / School of Applied Biology and Biotechnology, Department of Biotechnology / Greece

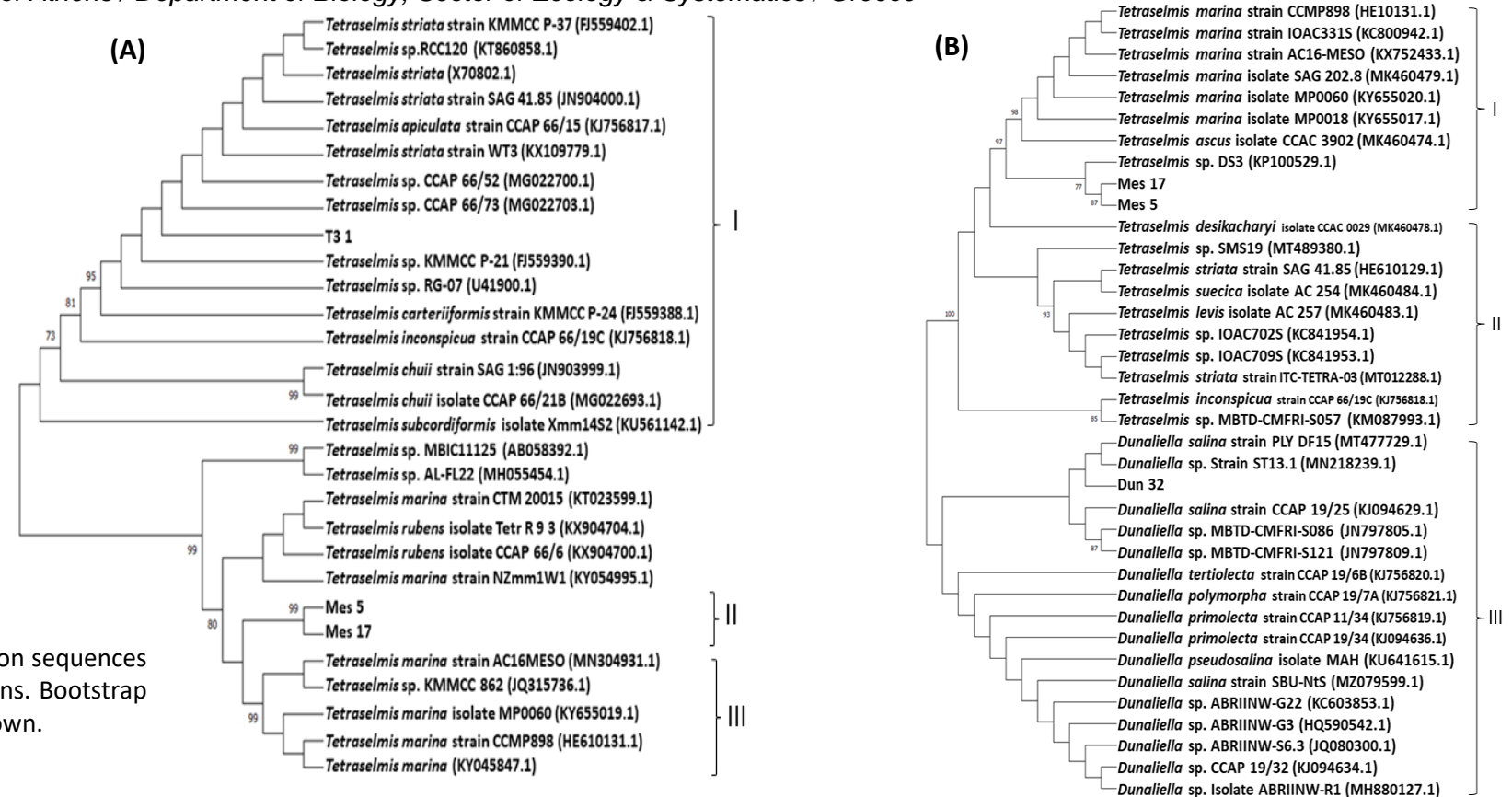
<sup>2</sup>National Institute of Research and Development for Biological Sciences / Institute of Biological Research Iasi, Department for Experimental and Applied Biology / Romania

<sup>3</sup>National & Kapodistrian University of Athens / Department of Biology, Sector of Ecology & Systematics / Greece

### Scope of the study

- AthU-AI (Athens University Algae) culture collection of the NKUA preserves strains isolated from various Greek coastal lagoons
- Strains studied: AthU-AI Mes5, AthU-AI Mes17, AthU-AI Dun30, AthU-AI Dun31, AthU-AI Dun32 and AthU-AI T\_3\_1
- Molecular markers: nuclear ITS region and 18S rDNA, plastid *rbcL* and *tufA*
- *TufA* and *rbcL* markers were selected to generate a phylogenetic tree based on their concatenated sequences

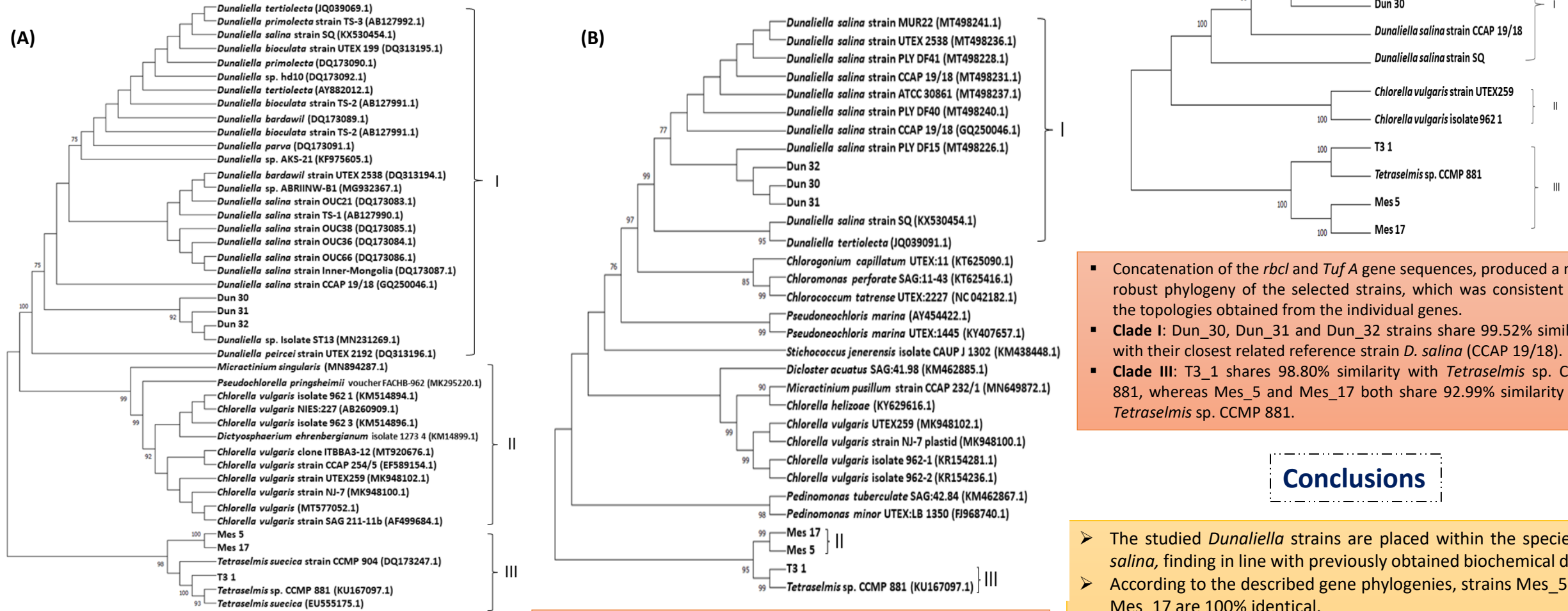
**Fig 1.** Maximum likelihood trees of **(A)** 18S rDNA and **(B)** ITS region sequences showing the phylogenetic relationship of studied microalgal strains. Bootstrap confident levels (based on 1000 replicates) below 70% are not shown.



- **Clade I:** T3\_1 shares 98.77% to 99.78% similarity with the reference strains comprised in the clade.
- **Clade II:** Mes\_5 and Mes\_17 share 99.78% similarity between them and form a separate lineage, in the vicinity of their closest relative *T. marina* NZmm1W1.

- **Clade I:** Mes\_5 and Mes\_17 strains are 100% identical and share 92.99% similarity with *T. desikacharyi*.
- **Clade III:** Dun\_32 share 99.42% similarity with its closest relative *D. salina* strain PLY DF15.

**Fig 2.** Maximum likelihood trees of (A) *rbcl* and (B) *TufA* genes sequences showing the phylogenetic relationship of studied microalgal strains. (C) Concatenated tree of the genes *rbcl* and *TufA*. Bootstrap confident levels (based on 1000 replicates) below 70% are not shown.



- **Clade I:** Dun\_30, Dun\_31 and Dun\_32 are 100% identical, and cluster together with *Dunaliella* sp. isolate ST13.
- **Clade III:** T3\_1 shares 98.78% similarity with its closest relative *Tetraselmis* sp. CCMP 881. Mes\_5 and Mes\_17 are 100% identical and cluster together within the clade, in a separate lineage.

- **Clade I:** Dun\_30, Dun\_31 and Dun\_32 share 100% identity with several *D. salina* strains included in the group.
- **Clade II:** Identical Mes\_5 and Mes\_17 strains are placed in a branch, in the vicinity of *Tetraselmis* sp.
- **Clade III:** T3\_1 shares 97.87% similarity with its closest reference strain *Tetraselmis* sp. CCMP 881.

- Concatenation of the *rbcl* and *TufA* gene sequences, produced a more robust phylogeny of the selected strains, which was consistent with the topologies obtained from the individual genes.
- **Clade I:** Dun\_30, Dun\_31 and Dun\_32 strains share 99.52% similarity with their closest related reference strain *D. salina* (CCAP 19/18).
- **Clade III:** T3\_1 shares 98.80% similarity with *Tetraselmis* sp. CCMP 881, whereas Mes\_5 and Mes\_17 both share 92.99% similarity with *Tetraselmis* sp. CCMP 881.

## Conclusions

- The studied *Dunaliella* strains are placed within the species *D. salina*, finding in line with previously obtained biochemical data.
- According to the described gene phylogenies, strains Mes\_5 and Mes\_17 are 100% identical.
- Phylogenetic analysis revealed that, within the genus *Tetraselmis*, exist complex and unresolved relationships indicating the need for a more in-depth investigation of unidentified strains with a multi-gene approach.